BIOCHEMICAL PARAMETERS OF TYPICAL CHERNOZEM SOIL UNDER SUNFLOWER AND VETCH+OATS IN CROP ROTATION WITH DIFFERENT FERTILIZATION

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Abstract

The aim of research was to compare soil biological properties under three fertilization systems in crop rotation.. The objective was to evaluate the impact of organic fertilizers in crop rotation on enzymatic activities representative of some steps of biogeochemical nutrient cycles: C(invertase), N(urease), P(phosphatase), and general microbial activity (basal soil respiration, ammonification capacity, dehydrogenase activity) in comparison to mineral and mixed fertilization. It was shown, the majority of biochemical parameters studied was reduced in soil under mixture vetch+oats followed sunflower in crop rotation, though the tendencies of change were similar. The enzyme activities were expressed per unit of soil organic carbon and it didn't change the previous conclusion: the urease and phosphatase activities increased, but the invertase activity reduced in soil fertilized by manure in comparison to NPK amendment. Soil basal respiration at field under sunflower was significantly lower ($P<0,05$) in soil amended by manure in comparison to one fertilized by NPK. Nitrogen mineralization capacity (ammonification) values were highly variable and do not allowed to reveal significant differences among treatments. Soil dehydrogenase activities of soil samples under both studied crops have shown the lower values at mineral fertilization. Our data confirm the assertion that the organic farming has the favorable impact on the chernozem soil biological properties.

Key words: crop rotation, fertilization systems, soil biochemical parameters, sunflower

Under the modern concept the soil quality is the ability to sustain plant and animal productivity, to increase water and air quality, and to contribute plant and animal health (Doran, Zeiss, 2000; Emnova, 2004). Although all physical, chemical, biological and biochemical properties are involved in soil functioning, biological and biochemical properties tend to react most rapidly to changes in the external environment, and are therefore generally used in estimating soil quality (Nannipieri et al., 1990; Trasar-Cepeda et al., 2008). Biological status of soil, is often proposed as a sensitive indicator of soil ecological stress or restoration process of the biological properties of arable land (Dick, Tabatabai, 1993; Dick, 1994).

Enzymes have an important role in nutrient cycles in soil. Soil enzymatic activity is conditioned by the activity of extracellular enzymes released from the roots and plant debrises, the microbial and soil animal cells, which are adsorbed on the surface of soil particles. In addition the soil enzymatic activity is driven by intracellular enzyme activity of soil organisms.

It is already accepted that soil enzymes can serve as a "fertility index" (Scujins, 1978) and enzyme activity is permanently inserted in the set parameters of soil quality as an indicator of the effectiveness of farming system (Dick et al., 1996; Aon et al, 2001). The data have been published, the organic farming system enhances microbial activity and enzyme status of arable soils (Liu et al., 2007; Senikovskaya, 2007). However, relations between soil enzymatic activity and supply of plants with mobile nutrients are little-studied. It should be mentioned, the enzymatic activity does not always correlate with soil microbial biomass, this highlights the role of plant extracellular enzymes and their dependence on plant species, and organic substance amended into the soil as fertilizer (Badiane et al., 2001).

The objective of this research was to assess the impact of organic fertilization in crop rotation system on the soil enzymatic activity, representing major stages of the biogeochemical cycles of nutrients in the soil, such as: C (invertase), N (urease), P (phosphatase), and overall microbial activity (basal soil respiration, ammonification capacity, dehydrogenase activity) compared with mineral and organo-mineral fertilizers. In our research we compared the soil biochemical variables in two fields: the cultivation of green mass mixture of vetch+oats along behind the sunflower both under three fertilization systems - organic, mineral and organo-mineral.

MATERIAL AND METHOD

Long-term experiment of field crops was established in 1971 in Balti steppe (North of Moldova Republic) on typical chernozem soil. Since 1991, three fertilization systems are studied in crop rotation: Mineral **(**MF) - NPK (130 kg active substance ha^{-1} of rotation surface), organic (OF) - cattle manure (15 t ha^{-1} of rotation surface), and mixed - the sum of indicated amounts of mineral and organic fertilizers (MOF).

Soil dehydrogenase activity (DH) (EC 1.1.1.1) was determined by a modified method of Galstean (1978). Urease activity (Ure) (EC 3.5.1.5) was measured by the method of Khaziev (1990). Alkaline phosphatase activity (AlkP) (EC 3.1.3.1) – by the method of Tabatabai and Bremner (1969). Invertase activity (**Inv**) (EC 3.2.1.26) – by a modified method of Ciunderova (1971) and Galstean (1978).

Soil basal respiration (SBR) was determined by the method proposed by Isermeyer (1952) and taking into account changes introduced by Dilly and Nannipieri (2001) after incubation of soil samples (10 g of each) for 7 days at 21°C, with optimum water content. The amount of CO_2 released during incubation, and interacted with NaOH, was determined by titrimetry. Nitrogen mineralization capacity (ammonification) (NMC, N-NH_4^+) was determined simultaneously with SBR analysis. From soil samples taken both before as well as those after incubation, ammoniacal nitrogen was extracted with 0.05 N NaCl solution (soil: solution ratio of 1:30). The quantity of ammoniacal nitrogen (N-NH_4^+) was determined using Nessler reagent (Mineev, 1989). NMC (mg N-NH_4^+ g $soil^{-1}$ day^{-1} 21°C) was calculated from the difference between values obtained in samples after and before incubation.

Total organic carbon (TOC) content was determined in air-dried soil samples by wet oxidation with potassium dichromate in an acid environment, and then by dosage of remained potassium dichromate excess by use the method of Tiurin (Arinushkina, 1970). pH values were measured using a glass electrode, based on the ratio of soil to 1 M KCl solution 1:2.5 g / ml (weight: volume).

Mobile ammoniacal nitrogen (N-NH_4^+) from typical chernozem was extracted with 0.05 N NaCl solution (1:30 ratio of soil to solution) and subsequently measured with Nessler reagent (Mineev, 1989).

Inorganic phosphorus (P_i) was extracted with 0.5 N acetic acid (1:25 ratio of soil to solution) according to method proposed by Ciricov (Mineev, 1989) and then was determined by the method of Murphy and Riley (1962). Water content of soil and absolutely dry soil mass were determined immediately after sampling by drying for 6-24 hours at 105°C.

Data analysis was performed by use of Microsoft Excel for Windows XP (Microsoft Office). Matrix were analyzed with the same dimensions. Mean values were analyzed by Student t-test (bilateral test, type 3 with unequal variances) (Aon et al., 2001) at the P< 0.05 level.

RESULTS AND DISCUSSIONS

General biochemical parameters. Soil basal respiration (SBR) and *nitrogen mineralization capacity* (NMC, ammonification, N-NH_4^+) provides a general characterization of biological processes of organic matter mineralization in arable soil.

SBR indicates the rate of degradation of soil organic matter. This parameter was significantly increased (P <0.05) in both, soil fertilized with NPK (MF), as well as with mixed fertilization system (**MOF**) (*fig. 1*) compared with manure amendment (**OF**). The effect was more pronounced in soil under sunflower (row crop) than in soil under mixture vetch+oats. However, the quantities of CO_2 removed from soil collected under vetch+oats, subjected to both, residual manure (OF), as well as residual mixed mineral and organic fertilizers (**MOF**), remained at the level observed in soil under sunflower. The results confirmed the loss of less organic matter in soil fertilized with OF, and the activation of organic carbon decomposition by annual administration of NPK.

NMC values were highly variable and have not revealed significant differences between the fertilization systems (*fig. 1*). However, nitrogen mineralization processes differ depending on soil fertilization systems. Ammoniacal N content formed after incubation in soil with only organic fertilizers, (**OF**) showed the same level under sunflower and vetch+oats. Quantities of ammoniacal N in soil fertilized with MOF were the highest in soil under sunflower and unexpectedly very low in soil under vetch+oats. In soil with NPK fertilization (MF**)**, ammoniacal N content decreased from 1.91 under sunflower to 1.34 mg N-NH_4^+ g $soil^{-1}$ day^{-1} 21°C under vetch+oats. Further research is needed for more detailed conclusions.

Dehydrogenase activity (**DH**), strictly intracellular enzyme activity, is used to assess the general activity of soil microorganisms. Analysis of soil samples under both crops studied showed lower DH values with mineral fertilization and largest with organic and mixed fertilization (f*ig. 1*). The differences were significant versus mineral fertilization system only for sunflower, when the data were too variable for soil under vetch+oats. Thus, organic fertilization contributes to activation of soil microbial communities due to the addition of nutrients.

Specific biochemical parameters. Urease activity (Ure) showed the same trend in both soil samples under the studied crops (*fig. 2*). Lower Ure activity was observed in mineral fertilization, and significantly higher values were recorded in organic and organo-mineral fertilization. For the last two fertilization systems Ure activity in soil under sunflower was significantly higher as compared with the vetch+oats, which followed the sunflower. Thus, manure amendment in soil under sunflower activated urease activity and urea decomposition. These significant changes probably are caused by changes in microbial community structure due to organic fertilization system noticed earlier by the another researchers (Schloter et al., 1998).

Alkaline phosphatase activity (AlkP), only in soil under sunflower with mineral fertilizers was significantly ($P<0.05$) lower compared with soil amended with organic and mixed fertilizers (*fig. 2*). AlkP activity in soil under the vetch+oats varied slightly at different fertilization systems. This may be due to a sufficient amount of phosphorus in the studied soil.

Invertase activity (Inv) varied from 727 mg Glu g^{-1} $soil^{-1}$ h^{-1} 37°C in soil under sunflower with mineral fertilization, to the levels significantly lower - 691 and 658 mg Glu g $soil^{-1}$ h^{-1} 37°C in soils with organic and organo-mineral fertilizers, respectively (*fig. 2*). The trend was the same in soil under vetch+oats, ranging from 630 to 592 and 563 mg Glu g^{-1} $soil^{-1}$ h^{-1} 37°C, respectively. Increased invertase activity in soil fertilized with NPK correlated with increased SBR parameter at the same system of fertilization, and confirms the stimulating effect of NPK on the soil organic matter (SOM) decomposition.

Agrochemical parameters. The top layer of soil (0-20 cm) fertilized with NPK had a significantly lower content in soil organic matter (SOM) in both crops studied compared with organic fertilization and mixed system, which coincides with previously published data (Stadnic, 2006). Differences in pH values of soil samples were insignificant (*fig. 3*). Concentrations of nutrients (ammoniacal N and inorganic P) were significantly lower in the upper layer of soil under the vetch+oats compared to soil under sunflower.

Also, at the moment of soil sampling the water content in top layer was lower under vetch+oats, whatever the type of fertilization. This could be explained by differences in root system structure of plants studied. The vetch and oat roots are located usually at the top layer of soil and thus water and nutrient absorption occurs faster. The roots of sunflower up-take the water and nutrients mainly from deeper soil layers.

Thus, the biological activity of typical chernozem depends on fertilization system and crop species. Organic fertilization system worked most favorable on the biological properties of soil, namely the enzymatic activities. Soil biochemical activity correlated with agrochemical status, i.e. with the content of mobile forms of nitrogen and phosphorus. It was interesting to observe how it influences the crop yield.

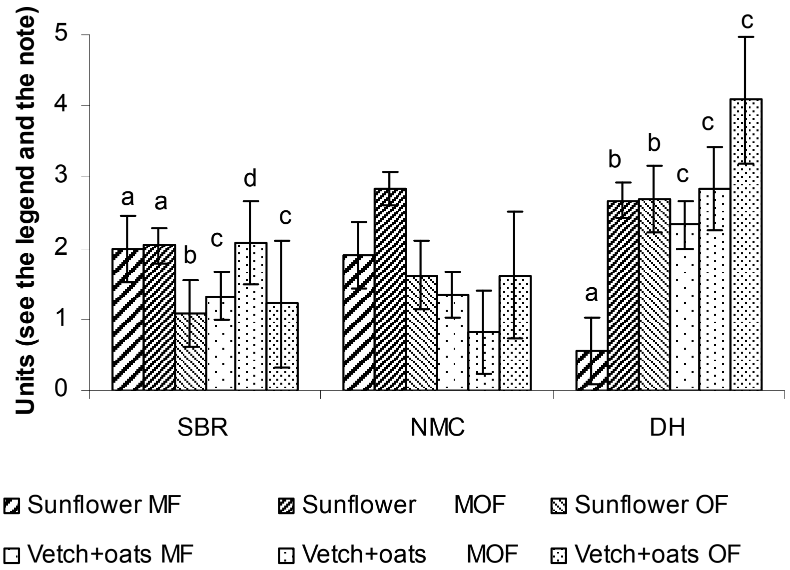

Figure 1 **General biochemical parameters of typical chernozem**
SBR – Soil Basal Respiration; NMC – Nitrogen Mineralization Capacity ; DH – Dehydrogenase activity

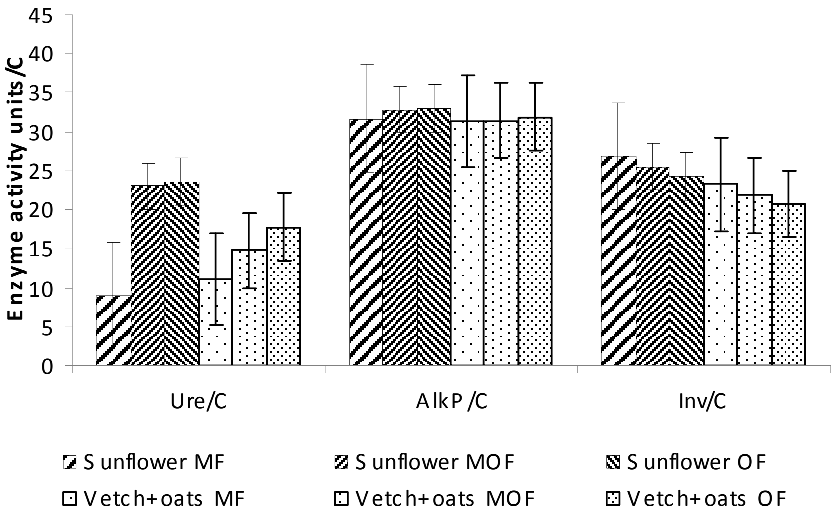

Figure 2 **Specific biochemical parameters of typical chernozem**
Ure – Urease activity; AlkP – Phospatase activity alcalină; Inv – Invertase activity

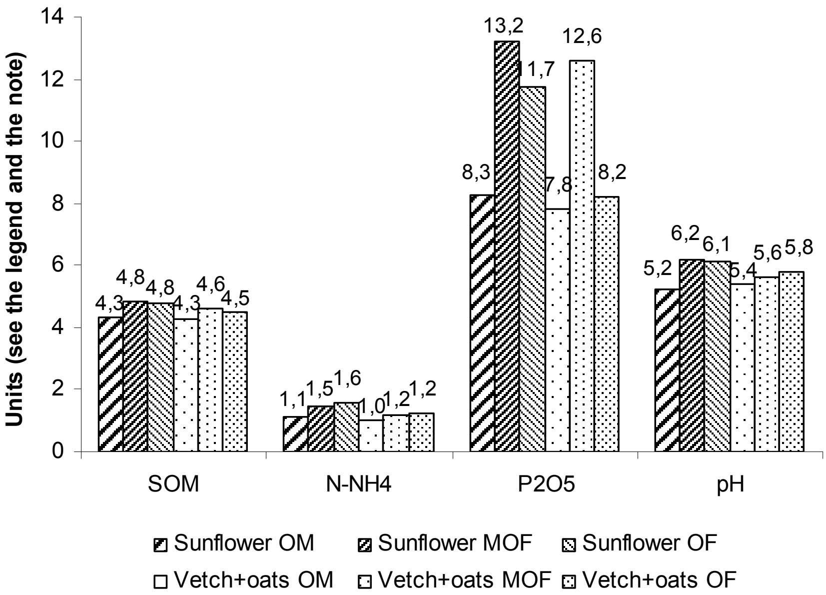

Figure 3 **Agrochemical parameters of typical chernozem**
SOM – Soil Organic Matter; N-NH_4 – ammoniacal N; P_2O_5 – inorganic P

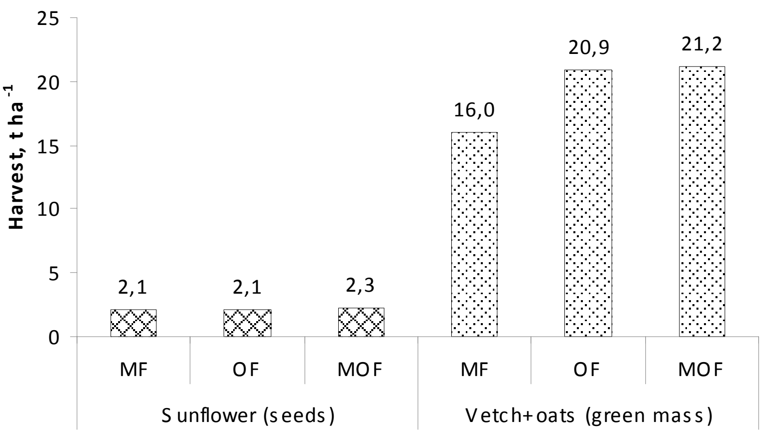

Figure 4 **Yield (t ha^{-1}) of sunflower and vetch+oats in crop rotation with different fertilization systems**

Yield of sunflower and vetch+oats in crop rotation. The overall ecosystem productivity and the productivity of crops in agricultural ecosystem is among the important criteria of soil health and quality. The data of sunflower yield show that indicated crop depends on soil fertilization system very insignificant (*fig. 4*). Only mixed fertilization system showed positive trend in yield increasing. These results are confirmed over several years (Nica, 2008; Boincean, 2008).

On the contrary, the yield of mixture vetch+oats increased significantly with manure amendment (34%) compared with using only the mineral system.

However, the hypothesized supposition that the low reacting of sunflower on fertilizer amendment is provoked by unknown restrictions for soil biochemical (microbiological) processes which deal with nutrient availability for plant should be investigated more deeply in the limits of the whole crop rotation (6 crops). The important observation for top soil layer (0-20 cm) under vetch+oats is significantly lower soil organic matter content, this fact requires especial attention to technology of sunflower cultivation in crop rotation.

Correlations between biochemical and chemical variables. The analysis of correlations performed separately for both selected crops and for each of system fertilization has shown a complicate picture regarding to the presence or absence of correlations, and the change from positive to negative correlations. In soils under sunflower (cultivated after spring barley) **BSR** in 2 treatments of 3 did not correlated with **SOM**. Only at **OF** was revealed poor but significant negative correlation (r = -0.49). In soils under vetch and oats (cultivated after sunflower) the **BSR** and **SOM** have shown the high positive correlation at **OF** (r = 0.86), and poor negative correlation at **MF** (r = - 0.52). The ammonification process positively correlated with **SOM** under both crops at **MF**, only, respectively, r = 0.90 under sunflower, r = 0.69 under vetch and oats. **DH** activity correlated with **SOM** in soils under sunflower, only. The **Ure** and **AlkP** activities as a rule correlated with **SOM**, but the relationship changed from positive to negative correlations. **Inv** activity did not correlated with **SOM** in all studied soils.

Good positive correlation between **pH** values and **DH** activity was observed at **MF** and **OF** under both crops, as well as for **Ure** activity under sunflower. The nutrient components: ammoniacal **N** and inorganic **P** - positively correlated with **Ure** and **AlkP** activities in most treatments, while, in general, soils amended by organic fertilizers were not characterized by increase of correlations between biochemical and chemical parameters under both crops.

Biochemical properties were influenced significantly by soil water content more often at **MF**, at that, the most correlations were negative under sunflower, and positive under vetch+oats.

Correlations between general and specific biochemical parameters. The mutual correlations between general and specific biochemical parameters within fertilization systems and cultivated plants did not revealed strict regularity, especially in soils under sunflower. At that, just in soil under sunflower at **OF** system the specific biochemical parameters were highly positively correlated each other. Besides that, it can be noticed under mixture vetch and oats an increase of correlations between all biochemical parameters at **OF** and especially at **MOF**.

Data obtained in the present research show that three types of soil fertilization generates significant changes in biochemical properties of typical chernozem. Values of biochemical parameters were lower in soil amended with mineral fertilizers versus the ones in soil fertilized with organic and mixed mineral+organic fertilizers for both crops studied. These significant changes probably are caused by changes in microbial community structure due to difference in fertilization system. Values of most biochemical parameters investigated were lower in soil under vethc+oats following sunflower in rotation. This fact may be explained by biological feature of sunflower to absorb nutrients from deep soil reserves, including from soil organic matter. Therefore, soil fertilization with manure just under sunflower has a positive impact on soil fertility for next crop vetch+oats following in the crop rotation. Biochemical and agrochemical properties of soil improves, and the productivity of vetch+oats increases. Thus, the organic fertilization system acts most favorable on soil biological properties, namely the enzymatic activities, which are the main stages of transformation of soil organic matter, including its labile fraction.

CONCLUSIONS

Of three system of fertilization studied, organic fertilization system work more favorable on biological properties of soil, namely the enzymatic activities, which are the main stages of transformation of soil organic matter, including its labile fraction. The specific biochemical parameters were highly positively correlated each other in soil under sunflower just at **OF** system.

The increase of correlations between all biochemical parameters at **OF** and especially at **MOF** was noticed under mixture vetch and oats. The hypothesized supposition that the low reacting of sunflower on fertilizer amendment is provoked by unknown restrictions for soil biochemical (microbiological) processes, which deal with nutrient availability for plant, should be investigated more deeply.

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